REMARKS

Upon entry of the above amendment, claims 1 and 16-28 will be pending in the application, claims 19-28 having been newly added. Support for the amendments to claims 16 and 17 can be found throughout the specification. For example, the language that appears in part (c) of claim 16 is supported in the substitute specification filed September 28, 2006, at page 3, lines 19-24 (which discusses immunizing the mouse and thereby producing an antibody). The new language that appears in part (b) of claim 16 is supported by claim 17 as originally filed. Also see page 10, lines 16-27, which discloses expression of a protein "on" baculovirus particles and that budding viruses (such as baculovirus) are "covered with an envelope." Page 11, lines 3-9, discusses expressing exogenous proteins "on the envelope of the baculovirus." Together, these statements clearly communicate that a target antigen, such as a protein, can be expressed on the surface of a baculovirus particle. (The specification says that "target antigen" includes categories such as proteins, sugar chains, and lipids (page 3, lines 33-35), so a "membrane protein" (i.e., a protein that would be imbedded in the viral envelope) is just one example.)

Support for new claims 19 and 23 can be found in original claim 18, as well as in the substitute specification, e.g., at page 10, lines 9-12.

New claims 20 and 22 are supported in the substitute specification at, for example, page 9, lines 20-36, which discusses, *inter alia,* a "portion" of a virus, such as a viral envelope portion, that is contaminated with a membrane protein as a background antigen. Baculovirus gp64 is described as a common contaminating background antigen at page 1, lines 25-32.

New claims 21 and 24 are supported in the substitute specification at, for example, page 3, lines 34-36.

Support for new claims 25 - 28 can be found, for example, at page 2, lines 29-35.

No new matter has been added.

Interview Summary

Applicants thank the Examiner for his helpful comments during the telephonic interview with the undersigned on November 22, 2010 (the "Interview"). The above new claims and certain proposed amendments to claims 16 and 17 similar but not identical to those specified above were discussed. Because the Examiner found claim 16 to be somewhat confusing, Applicants have reworded it as shown above; it is hoped that this improves the readability of the claim. Applicants are open to further suggestions.

The rejection for lack of enablement was discussed at length and the obviousness-type double patenting rejection was briefly discussed. As suggested by the Examiner, Applicants present their detailed arguments regarding both grounds for rejection in this Reply.

Applicants pointed out in the Interview that the pending Office action contained no ground for rejection of claim 1, yet did not acknowledge the allowability of this claim. During the Interview, the Examiner indicated that the rejections in the Office action did not apply to claim 1, and so claim 1 would be allowable. New claim 28 depends from claim 1, so presumably is also allowable. Applicants respectfully request that this be acknowledged in writing.

Presently Claimed Methods

As generally described in the present specification (see, e.g., the background section at pages 1-2), it is known in the art to produce recombinant proteins in insect cells *in vitro* by infecting the cells with a recombinant baculovirus encoding an exogenous protein. Where the exogenous protein is to be used as a target antigen to generate antibodies in an animal, the target antigen can first be at least partially purified from the baculovirus before introduction into the animal. Alternatively, the intact baculovirus with a target antigen on its surface can be used as the immunogen.

One problem with immunizing with antigens produced in baculovirus is discussed in the specification at pages 1-2. A major component of the baculoviral envelope is a baculoviral membrane protein, gp64, that is itself highly immunogenic. When gp64 is present as a component (e.g., a contaminant) of the target antigen composition used for immunizing an

animal, antibodies may be more readily produced against gp64 than against the target antigen. This can make it difficult to obtain the desired antibodies against the target antigen.

In an effort to reduce the immunogenicity of gp64 so that target antigens produced in a baculovirus system can be more usefully utilized as immunogens, even when contaminated with residual gp64, Applicants generated transgenic mice that are transgenic for soluble gp64, i.e., gp64 lacking a transmembrane domain. These mice are an improvement over mice transgenic for full-length gp64 (i.e., gp64 including the transmembrane domain; *see*, e.g., US Patent No. 7,750,204) in that male mice transgenic for full-length gp64 have been found to have a phenotype of inadequate testes development and sperm formation, so are infertile. By eliminating sequence encoding the transmembrane domain from the gp64 transgene utilized in the presently claimed transgenic mouse, the present inventors produced a transgenic mouse that did not have the infertility issues of the mice transgenic for full-length gp64, yet were still effectively immunotolerized to the gp64 present in baculovirus preparations. This immunotolerance means that the transgenic mice of the present claims produce a relatively low level, if any, antibodies to contaminating gp64 when exposed to target antigen compositions derived from baculovirus, thereby improving the production of antibodies to desired target antigens. See page 2, line 1, to page 3, line 1.

As specified in part (b) of claim 16, the "immunogen" of claim 16 can be a baculovirus particle or a portion thereof, and must comprise both gp64 and the target antigen. In an intact baculovirus particle, gp64 is an integral, natural part of the envelope. Lysing the baculovirus, and perhaps fractionating it to at least partially purify the target antigen, can produce a "portion" of a baculovirus particle containing both the target antigen and contaminating background antigens, including gp64. The present methods are useful whenever gp64 is present as a contaminant in the immunogen (along with the target antigen), as the present methods reduce the immunogenicity of the contaminating gp64 and thereby improve the production of antibodies against the target antigen. See the discussion at page 9, lines 16-36. The method of claim 16 encompasses use of either (i) an immunogen comprising a baculovirus particle comprising gp64 and the target antigen, where the target antigen is a protein expressed on the surface of the particle; or (ii) an immunogen comprising a portion of a baculovirus particle, where the portion

comprises gp64 and the target antigen. In the case where a "portion" is used, the target antigen could be, but need not have been, expressed on the surface of the particle from which the "portion" is derived—it could instead be, e.g., a soluble protein or any other antigen. The gp64 is typically present in the "portion" as a contaminant or as a part of a fusion protein with the target antigen. (See the discussion of fusion proteins below.) In an animal other than the transgenic animal of the present invention, the gp64 present in the immunogen would produce a problematic anti-gp64 immune response, interfering with production of antibodies against the desired target antigen. By using the gp64-containing immunogens in transgenic animals that are immunotolerant to gp64, such as the presently claimed transgenic animals, one can avoid this interference by gp64 antibodies.

Rejection for lack of enablement under 35 USC §112, paragraph 1

Claims 16-18 were rejected for lack of enablement. The Office action at page 2 acknowledges that the specification is enabling for

> a method for producing an antibody against a membrane protein comprising:
>
> (a) immunizing the transgenic mouse of claim 1 with a baculoviral particle, wherein the baculoviral particle comprises a nucleic acid sequence encoding a membrane protein, and wherein the membrane protein is displayed on the surface of the baculoviral particle;
>
> (b) recovering an antibody from the transgenic mouse, wherein the antibody recognizes the membrane protein.

The Office action goes on to state that the specification "does not reasonably provide enablement for immunizing the transgenic mouse with an immunogen comprising i) a baculoviral particle or portion thereof, and ii) a target antigen as broadly claimed." Applicants understand that the concerns expressed in the Office action are regarding enablement of the use of (1) a "portion" of a baculoviral particle, and (2) target antigens that are not membrane proteins. These two concerns, and some additional remarks in the Office action, are addressed below.

(1) Portion of a baculoviral particle

As discussed during the Interview, an immunogen containing a "portion" of a baculovirus particle includes, for example, a baculovirus lysate, or a partially or highly purified fraction derived from such a lysate. Claim 16 requires that both gp64 and the target antigen be present in the "portion"; as noted above, gp64 is commonly present as a contaminant in fractions derived from baculovirus, or as a component of a fusion protein in which the target antigen is recombinantly fused to gp64 (see further discussion of fusion proteins below). It is the presence of the interfering gp64 contaminant that makes the use of the presently claimed transgenic animals particular advantageous compared to use of wildtype mice, and that is why gp64 is specified as being present in the immunogen (not because gp64 is contributing anything useful to the immunogen). Applicants submit that use of a "portion" of a baculovirus particle as an immunogen is well within the ability of one of ordinary skill in the art. It is certainly well known to express recombinant proteins (e.g., target antigens) in a baculovirus/insect cell system. It is also well known to lyse the baculovirus to produce a lysate containing the target antigen as well as contaminating gp64, since gp64 is a common component of the baculovirus envelope. Such a lysate would be classifiable as a "portion" of a baculovirus particle. One of ordinary skill in the art is also well aware of standard methods to fractionate the baculovirus lysate in order to purify, at least in part, target antigens that were expressed in the baculovirus. If the purified target antigen composition contains some contaminating gp64, then it also qualifies as a "portion" of a baculovirus particle as recited in claim 16. For example, the "portion" might be a fraction enriched for viral envelope in which both the gp64 and the target antigen are imbedded, or might be a soluble fraction containing a soluble antigen plus some contaminating traces of viral envelope in which the gp64 is imbedded, or might be a fusion protein containing both gp64 and the target antigen. As long as the "portion" contains the target antigen, it will be presumptively useful for generating antibodies against the target antigen in the transgenic mouse. As Applicants understand it, the Examiner agreed during the Interview that one of ordinary skill in the art would be able to make and use a "portion" of a baculovirus particle comprising gp64 and a target antigen, as set forth in claim 16, such that this aspect of the claim is adequately enabled.

(2) Target antigen

The Office action suggests that, in the context of an immunogen that comprises an intact baculoviral particle, expression of the target antigen inside the particle, rather than on its surface, would mean the humoral immune system would not have access to the target antigen and so would not produce anti-target antigen antibodies. Applicants have addressed this concern by amending claim 16 to require that, when the immunogen comprises a baculovirus particle, the target antigen is a protein expressed on the surface of the particle. The Office action also opines that "the specification fails to teach how to immunize with a baculoviral particle expressing a non-membrane protein." Applicants submit that, so long as the target antigen is expressed on the surface of the baculoviral particle, whether it can be classified as a "membrane protein" or not is irrelevant. The art is well aware of how to display a target antigen on the surface of a baculoviral particle by expressing the target antigen recombinantly fused to a baculovirus protein such as gp64. See, for example, Lindley et al., J. Immunol. Meth. 234:123-135, 2000 (mentioned on pages 2-3 of the specification as "Non-Patent Document 2" and submitted as ref. BX in the Information Disclosure Statement filed October 4, 2007; a copy is attached hereto as Exhibit 1 for the Examiner's convenience). Lindley et al. describes production of monoclonal antibodies specific for human proteins LXRβ and FXR, using as the immunogen intact baculoviral particles displaying gp64 recombinantly fused to LXRβ or FXR. LXRβ and FXR are not membrane proteins; rather, they are soluble transcription factors found naturally in the nucleus of cells. See the UniProtKB/Swiss-Prot printouts from the Internet supplied as Exhibits 2 and 3. Lindley et al. demonstrates that those of skill in the art at the time the invention was made were aware that non-membrane proteins could be displayed on the surface of baculovirus by fusing the proteins to a surface protein such as gp64. Further evidence that this fusion protein technique works is shown in the post-filing date publication (by one of the present inventors and others) attached as Exhibit 4: Saito et al., Neuroscience Letters 465: 1-5, 2009. Saito et al. describe at page 2, carryover paragraph of col.1-2, the production of monoclonal antibodies specific for the non-

membrane[1] human protein Dj-1 by immunizing mice with baculovirus expressing a fusion protein containing a portion of the Dj-1 sequence fused to gp64. (See also the "Supplementary data" published by Saito et al. in the online version of the article; these "Supplementary data" are attached to the copy of Saito et al. at Exhibit 4.) These references show that the Examiner's concern that non-membrane protein target antigens cannot be expressed on the surface of baculoviral particles is unfounded. Applicants submit that one of ordinary skill in the art at the time of the invention would have been well aware of how to do it, and would have expected such baculovirus particles to be useful as an immunogen.

Additional remarks re: enablement

At page 4 of the Office action, the Examiner noted that "baculovirus is not administered at 1 mg/animal, for example (pg20, line 7); viral doses are measured in particle numbers or infectious units, not milligrams." The referenced passage at page 20, line 7, of the specification describes administration of a dose of an immunogen that is in the form of baculovirus particles. Applicants respectfully point out that immunogens are typically measured in milligrams or units of weight, and not in particle numbers or infectious units, even where the immunogen is in the form of virus particles. This is because the animals are not being "infected" with the baculovirus (which is an insect virus, not a mouse virus), but rather only immunized. See, for example, Lindley et al. at page 126, 2nd column, end of first paragraph, which details the total amount of antigen used for immunizations in terms of micrograms of total protein, and not particle numbers or infectious units. See also the Saitoh et al. article[2] cited at page 4 of the Office action. Saitoh et al. also measured their immunogen in terms of weight, not particle number or infectious units, noting at page 106, 2nd column, "For the immunization with BV [baculovirus], the *gp64 transgenic* mice were immunized subcutaneously with 1 mg of PepT1-BV in PBS and 100 ng of *pertussis* toxin." (Italicized text in the original.) PepT1-BV is baculovirus expressing the protein PepT1, just as in Example 4 of the present specification.

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[1] Saito et al. say at page 2, col. 1, second full paragraph, that Dj-1 is a "secreted" protein, which implies that it is not a membrane protein.

[2] Not to be confused with the Saito et al. article discussed above and attached as Exhibit 2.

Applicants wish to clarify the record regarding a comment on page 4 of the Office action, where Saitoh et al. is cited as teaching "sgp64 transgenic mice were immunized with PepT1...." In fact, Saitoh et al. did not use sgp64 (i.e., "soluble gp64") transgenic mice. Saitoh et al. used gp64 transgenic mice, i.e., mice transgenic for full-length gp64 (the mice disclosed in US Patent No. 7,750,204). Both the gp64 transgenic mice of Saitoh et al. and US Patent No. 7,750,204 and the sgp64 transgenic mice of the present invention are inherently immunotolerized to baculovirus gp64.

In view of the above, it is apparent that the specification enables one of ordinary skill in the art to practice the full scope of the claimed methods. Withdrawal of the rejection for lack of enablement is respectfully requested.

Rejection for nonstatutory obviousness-type double patenting

Claims 16-18 stand rejected as allegedly unpatentable over claims 1 and 2 of US Patent No. 7,750,204 (the '204 patent). According to the Office action, the conflicting claims are not patentably distinct form each other

> because [they] both require making antibodies against a target antigen using a transgenic mouse expressing gp64 that is immunotolerant to gp64 using a "baculovirus or portion thereof" (690) or a "budding virus or portion thereof" ('204)....Accordingly, the claims are not patentably distinct because the different species/genus within the claims are obvious variants and could have been claimed in either application.

The Office fails to note that the present method claims are limited to use of a mouse transgenic for gp64 "**wherein the gp64 is soluble and lacks a transmembrane region**." The specification of the '204 patent discloses creation of a mouse transgenic for full-length gp64. See Examples 1 and 2 at col.15-17. Neither the claims nor the specification of the '204 patent says anything about mice that are transgenic for soluble gp64. Nothing in the '204 patent would have provided a reason to alter the teachings of the '204 patent in order to prepare a gp64 transgenic mouse in which the transgene encodes less than the full length of gp64. Nor would there have been an expectation of success upon doing so. The present inventors made the surprising discovery that eliminating the transmembrane domain-encoding portion of the gp64 transgene improves the

fertility of the resultant male transgenic mice compared to their male counterparts transgenic for full-length gp64, while maintaining the benefit of immunotolerance to gp64 (including gp64 present in baculovirus-derived immunogens). This discovery was not predicted in the '204 patent. Further, contrary to the Office's position, the presently claimed methods could not have been claimed in the '204 patent, as they were not disclosed in that patent.

Applicants submit that present claims 16-18 are not obvious over claims 1 and 2 of the '204 patent. Furthermore, new dependent claims 19-27 add limitations that further distinguish the claims of the '204 patent. See, e.g., the limitations of claims 25-27, which require that the fertile mouse of claim 16, 17 or 20, respectively, is a male. Withdrawal of the rejection for obviousness-type double patenting is respectfully requested.

Conclusion

Applicants ask that the rejections be withdrawn and all claims allowed. If any issues remain, the Examiner is asked to telephone the undersigned to discuss.

The fee of $130.00 for the necessary extension of time is being paid concurrently herewith via the Electronic Filing System. Apply any other charges or any credits to deposit account no. 06-1050, referencing Attorney Docket No. 14875-0167US1.

Respectfully submitted,

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